

## ORIGINAL INVESTIGATION

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# Association of plasminogen activator inhibitor-1 and tissue plasminogen activator with type 2 diabetes and metabolic syndrome in Malaysian subjects

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## Abstract

**Background:** Increased plasma plasminogen activator inhibitor-1 (PAI-1) activity and decreased tissue plasminogen activator (tPA) activity could be considered a true component of the metabolic syndrome (MetS) associated with an increased risk of developing cardiovascular diseases (CVD) and fibrinolytic abnormalities. The aim of this study was to investigate the association of tPA and its inhibitor PAI-1 with type 2 diabetes (T2D) and MetS and interrelationship between PAI-1 and tPA activities and antigens in Malaysian T2D and normal subjects.

**Methods:** The plasma activities and antigens of PAI-1 and tPA and the levels of the tPA/PAI-1 complex as well as serum insulin, parameter of the coronary risk panel and plasma glucose at fasting state were studied in 303 T2D subjects (227 with MetS and 76 without MetS), 131 normal non-diabetic non-metabolic subjects and 101 non-diabetic MetS subjects.

**Results:** The PAI-1 activity was higher in subjects with T2D with MetS ( $P = 9.8 \times 10^{-19}$ ) and non-diabetic subjects with MetS ( $P = 3.0 \times 10^{-15}$ ), whereas the tPA activity was lower in T2D with MetS ( $P = 0.003$ ) as compared to normal subjects. Plasma tPA antigen levels were higher in subjects with T2D with MetS ( $P = 8.9 \times 10^{-24}$ ), T2D without MetS ( $P = 1.3 \times 10^{-13}$ ) and non-diabetic MetS subjects ( $P = 0.002$ ). The activity and antigen of PAI-1 in normal subjects were related to insulin resistance ( $P = 2.2 \times 10^{-4}$ ;  $0.007$ ). Additionally, the PAI-1 activity was associated with an increased waist circumference ( $P = 2.2 \times 10^{-4}$ ) and decreased HDL-c ( $P = 0.005$ ), whereas the tPA activity was associated with decreased FBG ( $P = 0.028$ ). The highest correlation was between PAI-1 activity and its antigen ( $R^2 = 0.695$ ,  $P = 1.1 \times 10^{-36}$ ) in diabetic subjects. The tPA activity negatively correlated with its antigen ( $R^2 = -0.444$ ,  $P = 7.7 \times 10^{-13}$ ) in normal subjects and with the PAI-1 activity and antigen ( $R^2 = -0.319$ ,  $P = 9.9 \times 10^{-12}$ ;  $R^2 = -0.228$ ,  $P = 3.4 \times 10^{-6}$ ) in diabetic subjects.

**Conclusions:** PAI-1 and tPA activities and antigens were associated with diabetes and MetS parameters in Malaysian subjects.

## Background

Central obesity, hyperglycaemia, high triglycerides (TG), low high-density lipoprotein cholesterol (HDL-c) and hypertension, all are well-documented risk factors for type 2 diabetes (T2D) and cardiovascular diseases (CVD) [1]. The constellation of these metabolic abnormalities

(known as a metabolic syndrome (MetS) or insulin resistance syndrome) increases the risk of T2D and CVD. The number of individuals with the MetS has increased globally during the past two decades, and this increase is associated with the worldwide epidemic of obesity and diabetes [2]. Obesity and physical inactivity are the driving forces for MetS and a person with MetS has 5-fold higher relative risk to develop diabetes [3] and 2.5 fold higher to develop CVD [4,5]. Overweightness and

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obesity lead to adverse effects on blood pressure, cholesterol, TG and impaired glucose tolerance (IGT) [6].

Plasminogen activator inhibitor-1 (PAI-1) is the primary physiological inhibitor of endogenous fibrinolysis that acts via inhibition of the tissue plasminogen activator (tPA) and the urokinase type activator (uPA), often leading to fibrin accumulation in basement membranes and interstitial tissues [7-9]. Elevations in plasma PAI-1 appear to compromise normal fibrin clearance mechanisms and promote thrombosis. The plasminogen activators (t-PA and u-PA) convert plasminogen to plasmin, which is involved in fibrinolysis, tissue remodelling and cell migration [10]. In addition to its role in intravascular fibrinolysis, PAI-1 is also involved in cell-associated proteolysis, cell migration, and tissue remodelling playing a role in pathological processes such as cancer cell invasion, metastasis and inflammation [11,12]. The majority of tPA in the blood is bound to its primary inhibitor, PAI-1 [13].

In large epidemiological studies elevated plasma PAI-1 has been demonstrated in various subgroups as an important feature of T2D and MetS [14-20] and this elevation may contribute to a thrombotic tendency [7-9,15,21]. This elevation precedes coronary artery disease [22] and even predicts the occurrence of first acute myocardial infarction and reinfarction [23-25]. Remarkably, the predictive ability of PAI-1 disappears after adjustment for markers of the MetS [26,27], suggesting that the MetS is a prerequisite to high plasma PAI-1 levels in patients prone to atherothrombosis. Moreover, it has been hypothesized that PAI-1 participates in the development of key features of the MetS. The circulating PAI-1 levels are positively associated with obesity and insulin resistance [28-30].

tPA activity may be an independent and early marker for asymptomatic lower extremity arterial disease in T2D [18,31]. Plasma tPA activities and the capacity of endothelial cells to secrete tPA in response to a fibrinolytic stimulus were also reported to be decreased in adults with diabetes [15]. Elevated plasma tPA antigens have been reported to be associated with insulin resistance, T2D, and obesity [14,18] and increased risk of CHD [32].

The aim of this study was to investigate the association of the plasma activities and antigens of PAI-1 and tPA, with T2D and MetS and to study the relationship between activities and antigens of PAI-1 and tPA.

## Materials and methods

### Subjects and data collection

This study involved diabetic patients with and without MetS and non-diabetic subjects with MetS receiving treatment at the University Malaya Medical Centre (UMMC), Kuala Lumpur. Normal subjects without

diabetes and MetS (the control group) in Klang Valley, Kuala Lumpur were recruited. The study was approved by the Medical Ethics Committee of University Malaya Medical Centre. Written informed consent was obtained from each subject. Patients with acute or chronic infections, severe medical conditions (malignancy, renal failure, liver cirrhosis, connective tissue disease, and chronic congestive heart failure) and pregnant women were excluded from the study.

Blood pressure (BP) measurements were taken from each patient's right arm in the seated position by using an Omron IntelliSense Automatic Blood Pressure Monitor after 10 min of rest in a quiet room in the morning. Two to three successive BP readings were obtained at 5-min intervals and averaged. Body weight and height were measured in the fasting state without shoes in the morning, and BMI was computed as weight in kilograms (kg) divided by height in meters squared ( $m^2$ ). Waist circumference was measured midway between the lower rib margin and the superior iliac spine at the end of gentle expiration in a standing position.

Fasting venous blood (10ml) was collected from each subject within a 2-hour window (8:00 to 10:00 AM) after 15 minutes rest, because of the diurnal variation of plasma PAI-1 [33]. The collected blood immediately taken into four labelled Vacutainer tubes, 0.109M trisodium citrate (for tPA and PAI-1 antigens and tPA/PAI-1 complex analysis), acidified 0.5M sodium citrate (for tPA and PAI-1 activities), sodium fluoride (for glucose measurement) and plain tubes (for insulin and lipid profile). The plasma/serum of trisodium citrate and plain tubes were separated gently within 30 minutes by using Allerga R X-12R centrifuge (Beckman Coulter, Inc US) for a 15 min at  $2500-3000 \times g$  at  $4^\circ C$ . Then the plasma/serum was separated into corresponding micro tubes and immediately kept at  $-80^\circ C$  until analysis.

### Biochemical Analyses

Serum TG, HDL-c and plasma glucose (FPG) were measured by an automated analyzer Dimension<sup>®</sup> RxL Max<sup>®</sup> Integrated Chemistry System. Insulin was measured by ADVIA Centaur assay XP Immunoassay System (Siemens Healthcare Diagnostics Inc. Deerfield, IL USA). All these investigations were done at Clinical Diagnostic Laboratory of the University Malaya Medical Centre (UMMC), Kuala Lumpur. Insulin resistance (IR) and Insulin sensitivity (IS) were calculated using the Homeostasis Model Assessment (HOMA2) Calculator v2.2 which is available from Oxford Centre for Diabetes, Endocrinology and Metabolism.

Plasma PAI-1 antigen was measured by TintElize<sup>®</sup> PAI-1 antigen ELISA test from Biopool (Trinity Biotech Inc. USA, Jamestown, NY). PAI-1 activity, tPA activity and antigen and tPA/PAI-1 complex were measured by

PAI-1 activity, tPA activity, tPA total antigen, tPA/PAI-1 complex human assays respectively, (Molecular Innovations, Inc. Peary Court, Novi, MI USA) according to the manufacturer's instructions. Plates were read at 450nm except the PAI-1 antigen plate which was at 490nm using a microplate reader (BioRad, USA).

### Statistical Analyses

The results were analyzed by the SPSS 11.5 (Social Package of Statistical Science) computer program by LEAD Technologies; Inc. USA). The missing data were listwise deleted (when any of the variables were missing, the entire observation was omitted from the analysis). The age, body mass index (BMI), waist, systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, HDL-c, FBS, insulin, IS, IR, PAI-1 and tPA activities and antigens and tPA/PAI-1 complex were log transformed because they were not normally distributed. These parameters means and 95% confidence intervals were transformed back and reported as geometric means. The association of PAI-1 and tPA activities and antigens and tPA/PAI-1 complex with T2D and MetS were assessed by univariate analyses (general linear model) adjusted for age, gender and race as covariates. The association of fibrinolytic PAI-1 and tPA activities and antigens with MetS parameters; (BMI, waist, SBP, DBP, TG, HDL-c, FBS, insulin, IS and HOMA (IR) (dependent variables) were analysed by hierarchical linear regression adjusted for age, gender and race as covariates in normal subjects. The interrelationship between fibrinolytic parameters; PAI-1 and tPA activities and antigens and tPA/PAI-1 complex in normal and T2D subjects were first evaluated by linear regression. This evaluation showed that (BMI, HDL-c and IR), (HDL-c and IR), (FBS), (Age and IS) and (BMI) were significantly associated with PAI-1 activity, PAI-1 antigen, tPA activity, tPA antigen and tPA/PAI-1 complex respectively. Then the correlation of fibrinolytic parameters with each other re-evaluated by hierarchical linear regression controlled for those corresponding confounder in addition to age, gender and race.

### Results

Of five hundred normal subjects freely respondents, only 190 were recruited for the study. After biochemical tests and application of IDF criteria for MetS diagnosis [34], 131 subjects were revealed as normal without diabetes and MetS (the control group). One hundred and one subjects out of 142 participants under treatment for hyperlipidaemia and/or hypertension at University Malaya Medical Centre (UMMC) were diagnosed as non-diabetic MetS subjects. Three hundred and three subjects who were previously diagnosed as T2D were participated in this study. Of these, 227 had MetS and

76 did not have MetS as defined by the IDF criteria for MetS diagnosis.

The demography and biochemical parameters of the subjects are shown in table 1. The fibrinolytic parameters, PAI-1 and tPA activities and antigens and tPA/PAI-1 complex assessed by general linear model (univariate) are shown in table 2. In general, the plasma levels of PAI-1 activity and tPA antigens were higher in T2D patients compared to normal subjects ( $P = 1.7 \times 10^{-8}$ ,  $3.0 \times 10^{-24}$  respectively) whereas the PAI-1 antigen and tPA activity ( $P = 0.01$ ,  $0.03$  respectively) were lower in T2D. There was no difference in tPA/PAI-1 complex between T2D and control subjects. On the other hand, the plasma PAI-1 activity was higher in non-diabetic MetS ( $P = 3.0 \times 10^{-15}$ ,  $1.5 \times 10^{-18}$ ) and T2D with MetS subjects ( $P = 9.8 \times 10^{-19}$ ,  $1.8 \times 10^{-21}$ ) compared to normal and T2D without MetS subjects respectively. Nevertheless, there was no difference in PAI-1 activity between the normal and T2D without MetS subjects and between non-diabetic MetS and T2D with MetS subjects (Table 2). However, the PAI-1 antigen was not significantly higher in non-diabetic MetS and significantly lower in T2D without MetS ( $P = 2.2 \times 10^{-9}$ ) compared to normal subjects. The results further showed that PAI-1 antigen was lower in both T2D without MetS ( $P = 6.9 \times 10^{-13}$ ) and T2D with MetS ( $P = 0.02$ ) compared to non-diabetic MetS subjects. Besides, it was higher in T2D with MetS ( $P = 5.1 \times 10^{-10}$ ) compared to T2D without MetS subjects. The tPA activity was lower in T2D with MetS compared to normal, non-diabetic MetS and T2D without MetS groups ( $P = 0.003$ ,  $0.002$ ,  $6.7 \times 10^{-5}$ ) respectively. On the other hand, there were no differences in the tPA activities between the other groups. In contrast, the tPA antigen was higher in non-diabetic MetS, T2D without MetS and T2D with MetS compared to control group ( $P = 0.002$ ,  $1.3 \times 10^{-13}$ ,  $8.9 \times 10^{-24}$ ) respectively. In addition, the tPA antigen was higher in T2D without MetS and T2D with MetS compared to non-diabetic MetS group ( $P = 1.3 \times 10^{-6}$ ,  $1.3 \times 10^{-12}$ ) respectively. Further the results showed that tPA/PAI-1 complex was lower in the T2D without MetS compared to normal ( $P = 0.04$ ), non-diabetic MetS ( $P = 0.02$ ) and T2D with MetS ( $p = 0.001$ ) groups while there were no differences in the tPA/PAI-1 complex between the other groups.

The association of fibrinolytic variables (PAI-1 and tPA activities and antigens) with MetS parameters was evaluated in normal subjects, and the results are depicted in table 3. The activity and antigen of PAI-1 associated with increased insulin levels ( $b = 1.941$ ,  $P = 1.2 \times 10^{-4}$ ;  $b = 1.614$ ,  $P = 0.015$ ), insulin resistance ( $b = 1.393$ ,  $P = 2.2 \times 10^{-4}$ ;  $b = 1.318$ ,  $P = 0.007$ ), and decreased insulin sensitivity ( $b = -1.963$ ,  $P = 1.1 \times 10^{-4}$ ;  $b = -1.637$ ,  $P = 0.012$ ). Furthermore, the PAI-1 activity

**Table 1 Demography and Biochemical parameters among normal, non-diabetic metabolic syndrome and type 2 diabetes with and without metabolic syndrome groups**

Parameters		Normal (n = 131)	Non-diabetic MetS (n = 101)	Type 2 diabetes	
				without MetS (n = 76)	with MetS (n = 227)
Gender%	Male/	18.5/29.2	23.9/14.8	17.7/11.3	39.9/44.7
	Female				
Races %	Malay	25.9	17.5	13.7	43.0
	Chinese	36.1	22.1	14.8	27.0
	Indian	12.1	18.8	14.8	54.4
Age (yrs)		47.2(44.9-49.7)	52.1(50.0-54.3)	49.8(47.6-52.1)	51.4(50.5-52.4)
	p-value		<sup>a</sup> 0.003	<sup>a</sup> 0.349	<sup>a</sup> 0.002
Body Mass Index (kg/m <sup>2</sup> )		22.7(22.1-23.3)	27.2(26.4-28.0)	23.34(22.5-24.3)	29.8(29.2-30.3)
	p-value		<sup>a</sup> 3.8 × 10 <sup>-13</sup>	<sup>a</sup> 0.515, <sup>b</sup> 6.2 × 10 <sup>-10</sup>	<sup>a</sup> 3.8 × 10 <sup>-13</sup> , <sup>b</sup> 3.2 × 10 <sup>-6</sup> , <sup>c</sup> 3.8 × 10 <sup>-13</sup>
Waist circumference (cm)		79.2(77.4-81.1)	94.5(92.8-96.3)	82.9(80.7-85.2)	98.6(97.4-99.7)
	p-value		<sup>a</sup> 3.8 × 10 <sup>-13</sup>	<sup>a</sup> 0.02, <sup>b</sup> 4.2 × 10 <sup>-13</sup>	<sup>a</sup> 3.8 × 10 <sup>-13</sup> , <sup>b</sup> 0.006, <sup>c</sup> 3.8 × 10 <sup>-13</sup>
Diastolic Blood Pressure (mmHg)		80.0(78.4-81.5)	85.6(83.6-87.6)	79.3(77.1-81.5)	83.1(81.7-84.5)
	p-value		<sup>a</sup> 1.7 × 10 <sup>-4</sup>	<sup>a</sup> 0.966, <sup>b</sup> 2.3 × 10 <sup>-4</sup>	<sup>a</sup> 0.02, <sup>b</sup> 0.004, <sup>c</sup> 0.02
Systolic Blood Pressure (mmHg)		130(127-134)	139(136-143)	129(125-134)	136(134-139)
	p-value		<sup>a</sup> 0.002	<sup>a</sup> 0.965, <sup>b</sup> 0.002	<sup>a</sup> 0.02, <sup>c</sup> 0.02
Triglyceride (mmol/l)		1.08(1.00-1.17)	1.59(1.46-1.72)	1.38(1.19-1.57)	1.78(1.66-1.91)
	p-value		<sup>a</sup> 1.6 × 10 <sup>-6</sup>	<sup>a</sup> 0.02	<sup>a</sup> 3.6 × 10 <sup>-13</sup> , <sup>c</sup> 0.001
High Density Lipoprotein (mmol/l)		1.54(1.48-1.61)	1.26(1.20-1.31)	1.22(1.15-1.29)	1.15(1.11-1.18)
	p-value		<sup>a</sup> 1.2 × 10 <sup>-10</sup>	<sup>a</sup> 2.6 × 10 <sup>-12</sup>	<sup>a</sup> 4.8 × 10 <sup>-13</sup> , <sup>b</sup> 0.008
Fasting Blood Glucose (mmol/l)		5.02(4.93-5.10)	5.36(5.23-5.49)	8.05(7.41-8.74)	7.74(7.39-8.11)
	p-value		0.24 <sup>a</sup>	<sup>a</sup> 3.8 × 10 <sup>-13</sup> , <sup>b</sup> 3.8 × 10 <sup>-13</sup>	<sup>a</sup> 3.8 × 10 <sup>-13</sup> , <sup>b</sup> 3.8 × 10 <sup>-13</sup>
Insulin (pmol/l)		45.7(41.7-50.1)	77.3(69.6-85.8)	63.7(52.9-76.5)	120(110-131)
	p-value		<sup>a</sup> 7.3 × 10 <sup>-9</sup>	<sup>a</sup> 0.002	<sup>a</sup> 4.1 × 10 <sup>-13</sup> , <sup>b</sup> 5.0 × 10 <sup>-8</sup> , <sup>c</sup> 1.4 × 10 <sup>-12</sup>
Insulin resistance (IR)		0.91(0.83-1.01)	1.53(1.37-1.69)	1.49(1.24-1.77)	2.59(2.38-2.81)
	p-value		<sup>a</sup> 1.0 × 10 <sup>-6</sup>	<sup>a</sup> 3.2 × 10 <sup>-5</sup>	<sup>a</sup> 4.1 × 10 <sup>-13</sup> , <sup>b</sup> 2.5 × 10 <sup>-12</sup> , <sup>c</sup> 3.8 × 10 <sup>-11</sup>

The result presented as geometric mean and 95% confidence interval of mean, <sup>a</sup>vs control group; <sup>b</sup>vs non-diabetic metabolic syndrome group; <sup>c</sup>vs type 2 diabetes without metabolic syndrome which evaluated by ANOVA. Bolt values are significant. MetS: metabolic syndrome.

associated with an increased waist circumference ( $b = 1.159$ ,  $P = 2.2 \times 10^{-4}$ ) and BMI ( $b = 1.199$ ,  $P = 0.001$ ) and decreased HDL-c levels ( $b = -1.125$ ,  $P = 0.005$ ). Although the tPA activity counteracts the PAI-1 activity which associated with decreased FBG ( $b = -1.079$ ,  $P = 0.028$ ), the tPA antigen showed no association with MetS parameters.

The interrelationship between activities and antigens of PAI-1 and tPA and tPA/PAI-1 complex parameters were further assessed in normal and T2D groups as shown in Table 4 and 5 respectively. Among normal subjects, the tPA activity negatively correlated with its antigen ( $r^2 = -0.44$ ,  $P = 7.7 \times 10^{-13}$ ) and weakly correlated with tPA/PAI-1 complex ( $r^2 = -0.090$ ,  $P = 0.001$ ). In contrast, the tPA antigen positively correlated with tPA/PAI-1 complex ( $r^2 = 0.202$ ,  $P = 3.5 \times 10^{-7}$ ). Unlike

normal subjects, there was a good correlation between fibrinolytic parameters in T2D, the PAI-1 activity well and positively correlated with its antigen ( $r^2 = 0.70$ ,  $P = 1.1 \times 10^{-36}$ ), and negatively correlated with tPA activity ( $r^2 = -0.32$ ,  $P = 9.9 \times 10^{-12}$ ). A similar correlation was shown between PAI-1 antigen and tPA activity ( $r^2 = -0.23$ ,  $P = 3.4 \times 10^{-6}$ ). The pattern of tPA antigen correlation with tPA/PAI-1 complex among T2D was similar to that in normal subjects, but it was a weak correlation ( $r^2 = -0.20$ ,  $P = 3.5 \times 10^{-7}$ ;  $r^2 = 0.06$ ,  $P = 7.9 \times 10^{-5}$ ).

## Discussion

The Association of fibrinolytic parameters with T2D and metabolic syndrome were studied; a higher PAI-1 activity is associated with T2D with MetS and MetS (nondiabetic), which is characterized by the presence of

**Table 2 Comparison of fibrinolytic PAI-1 and tPA between normal, non-diabetic metabolic syndrome, type 2 diabetes with and without metabolic syndrome and total type 2 diabetes groups**

Parameters	Normal (n = 131)	Non-diabetic MetS (n = 101)	Type 2 diabetes		Total Type 2 diabetes (n = 303)
			without MetS (n = 76)	with MetS (n = 227)	
PAI-1 activity (IU/ml)	12.8(11.1-14.7)	28.7(25.1-32.8)	11.0(9.37-12.9)	29.0(26.1-32.1)	21.7(19.7-23.9)
p-value		<sup>a</sup> <b><math>3.0 \times 10^{-15}</math></b> , <sup>c</sup> <b><math>1.5 \times 10^{-18}</math></b>	<sup>a</sup> 0.167	<sup>a</sup> <b><math>9.8 \times 10^{-19}</math></b> , <sup>c</sup> <b><math>1.8 \times 10^{-21}</math></b>	<sup>a</sup> <b><math>1.7 \times 10^{-8}</math></b> , <sup>b</sup> <b>0.02</b>
PAI-1 antigen (ng/ml)	30.2(27.1-33.7)	33.0(29.7-36.6)	18.2(16.2-20.6)	28.4(26.5-30.5)	25.4(23.9-27.1)
p-value		<sup>a</sup> 0.259	<sup>a</sup> <b><math>2.2 \times 10^{-9}</math></b> , <sup>b</sup> <b><math>6.9 \times 10^{-13}</math></b>	<sup>b</sup> <b>0.02</b> , <sup>c</sup> <b><math>5.1 \times 10^{-10}</math></b>	<sup>a</sup> <b>0.01</b> , <sup>b</sup> <b><math>4.7 \times 10^{-5}</math></b>
tPA activity (U/ml)	3.49(3.11-3.92)	3.51(3.14-3.91)	3.91(3.42-4.47)	2.82(2.58-3.07)	3.11(2.89-3.34)
p-value		<sup>a</sup> 0.965	<sup>a</sup> 0.206	<sup>a</sup> <b>0.003</b> , <sup>b</sup> <b>0.002</b> , <sup>c</sup> <b><math>6.7 \times 10^{-5}</math></b>	<sup>a</sup> <b>0.03</b> , <sup>b</sup> <b>0.03</b>
tPA antigen (ng/ml)	4.62(4.10-5.21)	5.99(5.35-6.70)	9.15(8.04-10.4)	9.85(9.14-10.6)	9.67(9.07-10.31)
p-value		<sup>a</sup> <b>0.002</b>	<sup>a</sup> <b><math>1.3 \times 10^{-13}</math></b> , <sup>b</sup> <b><math>1.3 \times 10^{-6}</math></b>	<sup>a</sup> <b><math>8.9 \times 10^{-24}</math></b> , <sup>b</sup> <b><math>1.3 \times 10^{-12}</math></b>	<sup>a</sup> <b><math>3.0 \times 10^{-24}</math></b> , <sup>b</sup> <b><math>1.7 \times 10^{-12}</math></b>
tPA/PAI-1 complex (ng/ml)	3.50(2.89-4.23)	3.57(2.97-4.30)	2.59(2.09-3.20)	3.91(3.46-4.42)	3.52(3.17-3.92)
p-value		<sup>a</sup> 0.879	<sup>a</sup> <b>0.04</b> , <sup>b</sup> <b>0.02</b>	<sup>a</sup> 0.336, <sup>c</sup> <b>0.001</b>	<sup>a</sup> 0.94

The result presented as geometric mean and 95% confidence interval of mean adjusted for age, gender and race, <sup>a</sup>vs control group; <sup>b</sup>vs non-diabetic metabolic syndrome group; <sup>c</sup>vs type 2 diabetes without metabolic syndrome which evaluated by univariate (General Linear Model). Bolt values are significant. MetS: metabolic syndrome.

abdominal obesity and insulin resistance; this result in agreement with other studies [35-40]. The plasma PAI-1 circulates in two states, active and latent. Active PAI-1 is bound to vitronectin (VN) [41,42] (the net molecular weight of PAI-1/vitronectin, ~125,000) while latent PAI-1 is unbound (MW ~50,000). The plasma vitronectin levels are increased in diabetes with nephropathy [43]. T2D subjects with renal failure were excluded from this study. However, nephropathy in T2D starts from the early stage of development diabetes. Thus the latent PAI-1 may be excreted in urine in quantities more than PAI-1/vitronectin complex, which may result in an increase in the ratio of active to latent PAI-1 in T2D compared to normal subjects. This may explain why

PAI-1 antigen is lower in T2D while PAI-1 activity is higher.

The Association of fibrinolytic parameters with MetS parameters was assessed in normal (control subjects), since the diabetic and non-diabetic MetS under treatment. There was a clear trend shows increasing of PAI-1 activity with increased insulin resistance, waist circumference, BMI, and decreased HDL-c. This would also explain the non-association of PAI-1 activity in subjects with T2D but without MetS.

PAI-1 is partly synthesised in fat cells, and its activity is related to abdominal obesity as mirrored by the high waist circumference [44]. In this regard, lipid infusion in normal subjects to the levels observed in T2D, and

**Table 3 Association of fibrinolytic PAI-1 and tPA with metabolic syndrome parameters among normal subjects (n = 131)**

Metabolic syndrome Parameters	PAI-1 activity b (P-value)	PAI-1 antigen b (P-value)	tPA activity b (P-value)	tPA antigen b (P-value)
Body Mass Index (kg/m <sup>2</sup> )	1.199 ( <b>0.001</b> )	1.079 (0.199)	-1.109 (0.104)	-1.021 (0.783)
Waist circumference (cm)	1.159 ( <b><math>2.2 \times 10^{-4}</math></b> )	1.057 (0.213)	-1.033 (0.496)	-1.035 (0.543)
Diastolic Blood Pressure (mmHg)	-1.038 (0.342)	-1.014 (0.763)	1.064 (0.185)	1.028 (0.607)
Systolic Blood Pressure (mmHg)	-1.009 (0.846)	1.007 (0.889)	1.074 (0.198)	1.084 (0.195)
Triglyceride (mmol/l)	1.119 (0.165)	-1.012 (0.899)	1.064 (0.558)	1.127 (0.331)
HDL- cholesterol (mmol/l)	-1.125 ( <b>0.005</b> )	-1.067 (0.166)	-1.076 (0.174)	-1.016 (0.807)
Fasting Blood Sugar (mmol/l)	1.047 (0.092)	1.079 ( <b>0.02</b> )	-1.079 ( <b>0.028</b> )	1.023 (0.583)
Insulin (pmol/l)	1.941 ( <b><math>1.2 \times 10^{-4}</math></b> )	1.614 ( <b>0.015</b> )	-1.236- (0.312)	1.288 (0.298)
Insulin Sensitivity (%)	-1.963 ( <b><math>1.1 \times 10^{-4}</math></b> )	-1.637 ( <b>0.012</b> )	1.265 (0.266)	-1.294 (0.289)
Insulin Resistance	1.393 ( <b><math>2.2 \times 10^{-4}</math></b> )	1.318 ( <b>0.007</b> )	-1.119 (0.305)	1.135 (0.313)

Result presented as b (P-value) assessed by linear regression, adjusted for age, gender and race. Bolt values are significant. b: coefficient for the relationship between the dependent variable "metabolic syndrome parameters" and the independent variable "fibrinolytic PAI-1 and tPA parameters". The positive sign of the coefficient implies a direct relationship, and the negative sign implies an inverse relationship.



**Table 4 Correlation between PAI-1 and tPA activities and antigens and tPA/PAI-1 complex parameters among normal group (n = 131)**

Parameters	Adjusted for	PAI-1 activity (IU/ml)	PAI-1 antigen (ng/ml)	tPA activity (U/ml)	tPA antigen (ng/ml)	tPA/PAI-1 complex (ng/ml)
<b>PAI-1 activity</b>	Age, gender, race, BMI, HDL-c & HOMA (IR)	<b>1</b>	0.085(0.973)	0.051(0.276)	-0.100(0.598)	0.035(0.552)
<b>PAI-1 antigen</b>	Age, gender, race, HDL-c & HOMA (IR)		<b>1</b>	0.044(0.60)	0.111(0.642)	0.029( <b>0.03</b> )
<b>tPA activity</b>	Age, gender, race & FBS			<b>1</b>	-0.444 ( <b><math>7.7 \times 10^{-13}</math></b> )	-0.090( <b>0.001</b> )
<b>tPA antigen</b>	Age, gender, race & IS				<b>1</b>	0.202( <b><math>3.5 \times 10^{-7}</math></b> )

Result presented as adjusted  $r^2$  (P-value) assessed by Linear regression. Bolt values are significant.

obesity resulted in increased PAI-1 concentration by 2 fold [45]. Dysfunctions in the fibrinolytic and endothelial system precede the development of overt T2D, which increases the risk of atherothrombotic disease long before overt diabetes is evident [45-47]. In addition, PAI-1 may predict T2D independent of insulin resistance and other known risk factors for diabetes, which may be due to increase in PAI-1 activity influenced by PAI-1 gene polymorphisms, adrenal steroids and angiotensin II activity [16,17]. The PAI-1 antigen levels were low in T2D with MetS and much lower in T2D without MetS. This finding can be partially explained by the high correlation between PAI-1 activity and its antigen in T2D (but not in normal subjects). On the other hand, PAI-1 activity was remarkably high in T2D with MetS but was normal in T2D without MetS. The high concentration of tPA antigen was mainly associated with T2D without effect of MetS parameters which in agreement with Eliasson et al, study [48]. The mechanism that explains this elevation of tPA antigen in T2D is yet unclear. The PAI-1 activity is negatively correlated with tPA activity, which may reflect the low tPA activity in T2D with MetS but not in T2D without MetS. High PAI-1 antigen inhibits tPA released from the vessel walls and decreased levels of free PA, *i.e.* low tPA activity [48].

The results of this study showed the increase in the concentration of tPA antigen was not paralleled by an

increase in free tPA. Therefore, plasma tPA activity was not increased and even declined modestly in T2D which in accordance with pervious study [49]. The low levels of plasma PAI-1 in T2D without MetS subjects may have reduced the combination of PAI-1 with free tPA. This leads to decrease the tPA/PAI-1 complex concentration in this group with no decline or simply an increase in free tPA levels.

MetS is estimated to be present in 75-80% of T2D patients and has been shown to influence CVD [50,51] and fibrinolytic function [52,53]. In patients with MetS but without diabetes, increased measures of atherosclerosis [54-56] and a higher incidence of cardiovascular events has been observed [52,57]. This has partly been attributed to impaired fibrinolytic function [52] due to altered regulation of PAI-1 and tPA [53,58,59]. However, treatment by a PAI-1 inhibitor improved histological remodelling of myocardium and arteries with suppression of inflammation and thrombus formation [60]. Metabolic- defining criteria were significant predictors for PAI-1 activity, while none of the diabetes related factors seemed to contribute to PAI-1 activity and tPA levels. Thus, the main inhibitor of fibrinolysis was found to be severely influenced by the state of MetS.

In some studies, decreased fibrinolysis, as measured by high PAI-1 activity, has been associated with cardiovascular events [61,62]. High levels of tPA antigen

**Table 5 Correlation between PAI-1 and tPA activities and antigens and tPA/PAI-1 complex parameters among type 2 diabetes group (n = 303)**

Parameters	Adjusted for	PAI-1 activity (IU/ml)	PAI-1 antigen (ng/ml)	tPA activity (U/ml)	tPA antigen (ng/ml)	tPA/PAI-1 complex (ng/ml)
<b>PAI-1 activity</b>	Age, gender, race, BMI, TG & IS	<b>1</b>	0.70( <b><math>1.1 \times 10^{-36}</math></b> )	-0.32( <b><math>9.9 \times 10^{-12}</math></b> )	0.038(0.132)	-0.078(0.773)
<b>PAI-1 antigen</b>	Age, gender, race, waist, TG & IS		<b>1</b>	-0.23( <b><math>3.4 \times 10^{-6}</math></b> )	0.024(0.08)	0.066(0.054)
<b>tPA activity</b>	Age, gender, race, BMI, TG & IS			<b>1</b>	-0.100( <b><math>5.1 \times 10^{-5}</math></b> )	-0.071(0.054)
<b>tPA antigen</b>	Age, gender, race & HDL-c				<b>1</b>	0.064( <b><math>7.9 \times 10^{-5}</math></b> )

Result presented as adjusted  $r^2$  (P-value) assessed by Linear regression. Bolt values are significant.

independently predict cardiovascular events both in a healthy population [62,63] and in patients with prevalent coronary disease [6,64]. Therefore, these results also support the hypothesis that several abnormal metabolic parameters are essential for the elevation of plasma PAI-1 and reduction of tPA activities since PAI-1 is a multifunctional protein. However, more studies are needed to further assess the association between PAI-1 expression and CVD.

## Conclusions

The PAI-1 activity was pronounced in MetS and T2D with MetS whereas the tPA antigen was an indicator for T2D without effect of MetS parameters. This impaired fibrinolysis leads to a suggestion that coronary heart disease seems to start tick in non-diabetic MetS before the onset of clinical diabetes. This was confirmed in quantitative trait analysis in which PAI-1 activity was associated with MetS parameters (waist, BMI, HDL-c and insulin resistance) which are well documented as risk factors for T2D and CVD.

## Abbreviations

BMI: body mass index; CHD: Coronary heart disease; CI: confidence interval; CVD: cardiovascular disease; DBP: diastolic blood pressure; FBS: fasting blood sugar; HDL-c: high density lipoprotein cholesterol; HOMA (IR): Homeostasis Model Assessment (Insulin resistance); IDF: International Diabetes Federation; IS: Insulin sensitivity; MetS: metabolic syndrome; PAI-1: plasminogen activator inhibitor-1; SBP: systolic blood pressure; TG: triglycerides; tPA: tissue plasminogen activator; T2D: type 2 diabetes; UMMC: University Malaya medical centre; uPA: urokinase plasminogen activator.

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## Authors' contributions

ZH collected data, performed practical and statistical analysis and drafted the manuscript; SA, performed statistical analysis, edited the manuscript and discussion. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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